https://doi.org/10.17221/17/2024-PPS

Effect of legume (Fabaceae Lindl.) seeds on selected life activities in the J2 stage of *Meloidogyne hapla*

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Citation: Dobosz R., Krawczyk R. (2024): Effect of legume (Fabaceae Lindl.) seeds on selected life activities in J2 stage of *Meloidogyne hapla*. Plant. Protect. Sci., 60: 193—206.

Abstract: *Meloidogyne hapla* is a serious pest of many cultivated plants. In response to the economic significance of the species, efforts are being made to develop a new method to reduce its harmful effects on crops. The aim of the study was to determine the effect of diffusates from seeds of selected species of legumes on the motility of second-stage juveniles and to evaluate the effect of meal from seeds of selected species of legume on the capacity to infect the roots of tomato plants by the J2 stage. The experiment examined the effect of diffusates on the motility of the J2 stage performed in Petri dishes, at temperatures of 10 °C, 17 °C and 21 °C. The evaluation of the J2 stage infectivity was estimated in a pot experiment performed under controlled conditions of 20 ± 1 °C. The pots were filled with sterile substrate mixed with meal from the seeds of selected plants at 1%, 5% and 10% of the substrate weight. The studies carried out in the Petri dishes showed varying effects of the seed diffusates from selected legume plants on the motility of the J2 stage of *Meloidogyne hapla*. J2 were found to lose their motility within 24 h after immersion in water containing seed diffusates from *Melilotus albus*, *Trifolium pratense T. repens*, in the temperature ranges investigated (10 °C, 17 °C and 21 °C). However, in a mixture of seed diffusates and soil filtrate from the root zone of tomato plants, the absence of motility in the second-stage juveniles was observed after 24 h at 17 °C and 21 °C, with seed diffusates from *Lotus corniculatus, Medicago sativa, Medicago* × *varia, Melilotus officinalis*, as well as *Onobrychis viciifolia, Ornithopus sativus, Vicia sativa*, used in the mixture. *Galega officinalis* Risa (GoR) seed diffusates were found to have an inhibiting effect on the motility of the J2 stage of *M. hapla* 24 h following the immersion of the J2 stage in the solution of the soil filtrate containing tomato root diffusates, at 21 °C. The J2 stage were not rendered immotile in all the experiment set-ups involving the seeds of *V. faba, Lupinus* spp., likewise in the control set-ups. In the pots studied, a significant effect of the addition of legume seed meal on the development of *M. hapla* nematodes and tomato plants was found. The introduction of *Lotus corniculatus, Onobrychis viciifolia* and *Vicia sativa* seed meal into the substrate in the proportion of 1%, 5% and 10% resulted in the inhibition of the J2 stage penetration into the roots of tomato plants at temperatures of 17 °C and 21 °C. With the admixture of the *M. sativa* and *T. repens* seed meal, within the temperature range investigated, no nematode infection was observed in the roots, regardless of the seed meal content in the substrate. As regards to the fresh weight, tomato plants grown in a substrate containing 1% and 5% of the *V. sativa* cv. Jaga seed meal were characterised by significantly higher plant weight values as compared to those grown in the control set-up. The obtained results imply that is advisable to expand the scope of research to include other economically important crops damaged by the northern root-knot nematode.

Keywords: *Meloidogyne hapla*; motility; infectivity; legumes; seed diffusates; seed meal

https://doi.org/10.17221/17/2024-PPS

Root-knot nematodes (*Meloidogyne* spp.) constitute a group of obligate plant parasites, which includes over a hundred species, several of which are of relevance as serious pests of crops. In tropical and subtropical regions, the most important parasitic root nematodes of the genus *Meloidogyne* include *M. incognita* (Kofoid and White, 1919), *M. javanica* (Treub, 1885), *M. arenaria* (Neal, 1889), M. *enterolobii* (Yang & Eisenback, 1983) and *M. graminicola* (Golden & Birchfield 1965). However, with regards to the regions which lie in a temperate climate zone, the greatest losses in crops are caused by *M. chitwoodi* (Golden et al. 1980) and *M. hapla* Chitwood, 1949 (Elling 2013).

The second-stage juveniles (J2 stage), the motile and infective stage of root-knot nematodes, attack the root tissues, where they undergo successive developmental stages and reach sexual maturity (adulthood). During this time, as a result of the plant-nematode relationship, reorganisation takes place in the roots of infected plants, leading to the formation of so-called giant cells, i.e., sites supporting the development of nematodes. As a result of the plant-nematode interaction, the genes involved in the plant defence response, as well the genes encoding the heat-shock proteins, cytoskeleton proteins and hormones regulating the developmental pathways, are expressed (Palomares-Rius et al. 2017; Oostenbreek et al. 2021). The root tissue changes result in decreased water uptake leading to a decrease in the leaf water potential, gas exchange parameters, stomatal conductivity, transpiration and photosynthesis manifested by wilting, stunting and even plant death (Strajnar et al. 2012). The growth inhibition or wilting observed in plants as a result of nematode feeding leads to a deterioration in the crop quality, as well plant product defects and a reduction in the yield at harvest, estimated at approximately several billion U.S. dollars (Elling 2013).

In response to the economic significance of root-knot nematodes in regions with a warmer climate, efforts are being made to develop innovative and effective, while safe for human health and the environment, management strategies to reduce their harmful effects on crops. Research is being conducted into the feasibility of using microorganisms, predators, industrial waste, by-products, animal manures and soil amendments with plant-derived materials obtained from different, both the above- and underground parts of plants of several plant families, for their effectiveness against root-knot nematodes (Renčo 2013; Baghaee Ravari & Mahdikhani Moghaddam 2015; Ntalli et al. 2020; Rosskopf et al. 2020).

Studies by Venkat Rao et al. (1986) and Bawa et al. (2014), Bello et al. (2007), Elbardi et al. (2008); Asif et al. (2014), Sahu et al. (2018), Akhtar et al. (2019), El-Nuby and Alam (2020) and Mendes-Lopes et al. (2020) found a significant effect of plants from the Fabaceae family (Fabace Lindl.) towards reducing the process of leaving eggs by second-stage juveniles of *M. incognita* and *M. javanica*. Furthermore, Shakeel et al. (2010) and Asif et al. (2014) showed that legume plants may have a limiting effect on the motility of the J2 forms of *M. incognita* and *M. javanica* as well as on their mortality. The application of leaf or root extracts of certain legume plants, i.e., *Leucaena leucocephala* Lam. De Witt and *Gliricidia sepium* (Jacq.) as well as the comminuted plant material of *Acacia arabica* (L.), *A. nilotica*, Jack bean *Canavalia ensiformis* (L.) seed -meal, *Pisum* sp. and alfalfa (*Medicago sativa* L.), locust bean (*Parkia biglobosa* L.), and *Millettia pinnata* (L.) reduced the root infections in tomato plants, lentil (*Lens culinaris*) Medik plants or the cowpea *Vigna unguiculata* L. by second-stage juveniles and contributed to suppressing the population growth of these nematodes (Morris & Walker 2002; Adekunle & Akinlua 2007; Lopes et al. 2009; Leonetti 2011; Ismail 2016; Eche & Okafor 2020; Ansari et al. 2020).

M. hapla is one of the most significant invasive root nematodes causing considerable economic losses as a result of reducing the quality and size of crops among others, the carrot *Daucus carota* L. (Gugino et. al. 2006; Douda et al. 2021), the tomato *Solanum lycopersicum* (L.) (Barker et al. 1976; Seid 2015), lettuce *Lactuca sativa* L. (Viane & Abawi 1996, 1998) and celery *Apium graveloens* L. (Kornobis 2003) in North America and Europe.

Currently, the rudimentary measures to reduce the harmfulness of *M. hapla*, in accordance with the principles of integrated pest management mainly amount to prevention based on the appropriate rotation of crops where non-host plants, such as cereals or maize, are the forecrops for at least 2–3 growing seasons. In addition, in preventive measures, it is important to control the dicotyledonous weeds throughout the crop rotation, as host plants for the northern root-knot nematode (*M. hapla*), which constitute a reservoir of populations of this nematode species, as well as trap crops (Vestergård 2019) and cover crops

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rattlepods *Crotolaria* L. (Wang et al. 2002) and Sudan grass (Viane & Abawi 1998). When these treatments are insufficient to combat *M. hapla*, nematicides, i.e., pesticides designed to combat nematodes, are used.

However, other, safer for the environment, solutions to combat *M. hapla* or reduce its harmfulness are still being sought. Hussain et al. (2017) showed a significant decrease in the size of the nematode population in a carrot crop following the application of *Lecanicillium muscarium*. However, the research by Hildago (2008), Douda et. al. (2010), Böhm et. al. (2009), Anita (2012), Dahlin and Hallmann (2020) and D'Addabbo et al. (2020) showed a nematocidal effect of fresh/alcohol extracts of selected Brassicaceae Juss species. After using these products, an increase in the mortality rate of second-stage juvenile invasive larvae and a reduction in the development of mature forms of the nematode were observed.

In view of the research on the possibilities to non-chemically reduce the occurrence of nematodes described in the literature and the importance of the northern root-knot nematode for plant production, mainly for food, the study was undertaken with the objective to: *(i)* determine the effect of diffusates from the seeds of selected species of legumes on the motility of the second-stage juveniles of *M. hapla; (ii)* evaluate the effect of meal from the seeds of selected species of legume plants, used by mixing with soil, on the capacity to infect the roots of tomato plants by the second-stage juveniles of *M. hapla*.

MATERIAL AND METHODS

Nematode culture

A population of the northern root-knot nematode was collected from a carrot field and identified to the species level based on the diagnostic protocols of Karssen (1999) and Petersen and Vrain (1996). The population was maintained under controlled conditions at a temperature of 20 ± 1 °C and day and night length of 16/8 h on tomato *Solanum lycopersicum* (L.) cv. Krakus, plants. The experiment used second stage juveniles that left the egg masses within 24 hours of the start of the experiment. The isolation of the larvae was performed using the modified method of Rodríguez-Kábana and Pope (1981).

Determination of the effect of seed diffusates (aqueous extracts) obtained from selected species of legumes on the motility of the J2 stage of *M. hapla*

Legume seed diffusates. The experiment examined the effect of diffusates (aqueous extracts) obtained by immersing 1 gram of legume seeds (Table 1) in 100 mL of two types of liquid for 24 h: *(i)* distilled water; *(ii)* soil filtrate from the root ball of tomato plants. The soil filtrate from the root ball of tomato plants was obtained from 4-week-old plants growing in a sterile soil substrate in 0.5 L pots. The nematode-free soil was subjected to a thermal treatment process. One-hundred millilitres (100 mL) of distilled water were applied to the tomato pots under optimal humidity conditions. To obtain 50 mL, the clear soil filtrate was collected (discarding the first 5 cm^3 of the filtrate).

Experiment. The effect of the diffusates from the seeds of selected species of legumes (Table 1) on the motility of the J2 stage of *M. hapla* was examined under four scenarios:

(i) J2 larvae + distilled water (control), *(ii)* J2 larvae + diffusates from the seeds in distilled water, *(iii)* J2 larvae + soil filtrate from the roots of tomato plants (control), *(iv)* J2 larvae + soil filtrate from the roots of tomato plants + seed diffusates in the soil filtrate.

In the tested cultivars, the motility of the J2 stage was assessed. Thirty (30) individuals of the J2 stage of *M. hapla* were placed in Petri dishes with a diameter of 3 cm filled with fluids from the tested cultivars. The Petri dishes were left at temperatures of 10, 17 and 21 ± 1 °C. The motility was assessed at two time intervals: after 24 and 48 h – the number of individuals that were rendered immotile were counted. The immotile nematodes were counted using stereoscopic microscope OLYMPUS SZ-6045 (Olympus, Japan) with a magnification of 40×. Two runs of three repetitions each were performed.

Evaluation of the effect of meal from the seeds of selected species of legume plants on the capacity to infect the roots of tomato plants by the J2 stage of *M. hapla* and on the growth of tomato plants

For this purpose, an experiment was performed under the controlled conditions of 20 ± 1 °C and day and night length of 16/8 h. Pots with a capacity of 0.25 L were filled with a sterile substrate (potting soil/gravel 1:1) mixed with meal from the seeds of selected plants, crushed in an impact grinder using a rotating knife, at 1%, 5% and 10% of the substrate weight. Soil sterilisation was carried out for 30 min at

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Table 1. List of abbreviations used in the text and in the graphs

EPPO code	English name	Scientific name
GAGOF	goat's rue (common goat's rue), cv. Risa	*Galega officinalis* L. cv. Risa
LOTCO	common birdsfoot trefoil, cv. Skrzeszowicka	*Lotus corniculatus* L. cv. Skrzeszowicka
LUPAL	white lupine	*Lupinus albus* L.
LUPAN	narrow-leaved lupine, cv. Oskar	*Lupinus angustifolius* L. cv. Oskar
LUPAN	narrow-leaved lupine, cv. Roland	*Lupinus angustifolius* L. cv. Roland
LUPLU	yellow lupine, cv. Parys	*Lupinus luteus* L. cv. Parys
MEDSA	Alfalfa (lucerne), cv. Blue Moon	*Medicago sativa* L. cv. Blue Moon
MEDVA	variegated alfalfa (sand lucerne), cv. Radius	*Medicago* × *varia* T. Martyn cv. Radius
MEUAL	white melilot (white sweetclover)	*Melilotus albus* Medik.
MEUOF	yellow melilot (yellow sweetclover)	*Melilotus officinalis* L. Pallas
ONBVI	common sainfoin (esparcet), cv. Taja	*Onobrychis viciifolia* Scop. cv. Taja
OROSA	serradella (birdsfoot)	*Ornithopus sativus* L. cv. Emena
TRFPR	red clover, cv. Nike	*Trifolium pratense* L. cv. Nike
TRFRE	white clover, cv. Grassland Huia	*Trifolium repens* L. cv. Grassland Huia
VICFM	field bean (horse bean), cv. Granit	*Vicia faba* L. ssp. *minor* cv. Granit
VICFM	field bean (horse bean), cv. Albus	*Vicia faba* L. ssp. *minor* cv. Albus
VICSA	common vetch (garden vetch), cv. Ina	*Vicia sativa* L. cv. Ina
VICSA	common vetch (garden vetch), cv. Jaga	*Vicia sativa* L. cv. Jaga
	Tested cultivars of juveniles of second stage (J2)	
J2+S+H_2O	juveniles of second stage immersed in seeds diffusates of plants in water	
J2+H_2O	juveniles of second stage immersed in water; control 1	
J2+S+RD	juveniles of second stage immersed in seeds diffusates of plants in root diffusates of *Solanum lycopersicum*	
J2+RD	juveniles of second stage immersed in root diffusates of *S. lycopersicum*; control 2	

121 °C and 205 kPa. After 24 h, 150 individuals of the J2 stage were introduced into the soil, and after 48 h, tomato plants at the 4–5 leaf stage (BBCH 14–15) were planted, which were previously weighed and their heights were measured. For a period of 21 days, the growth of the tomato plants occured in optimal moisture conditions. After 21 days, the plants were taken out and the roots were cleaned. The plants were weighed and their heights were measured, and the number of leaves was counted. The plant weight and height were presented as percentages. The roots were stained with acid fuchsin in lactoglycerine (Hooper 1986). The root galls and infections were counted using an OLYMPUS S2 6045 stereoscopic microscope with a magnification of 40×.

Statistical analysis

Data from the Petri dish and pot experiments were subjected to an analysis of variance analysis (ANOVA). The significance of the differences between the means was assessed by Fisher's test at a level of $P \leq 0.05$.

RESULTS

Determination of the effect of the seed diffusates obtained from the selected species of legumes on the motility of the J2 stage of *M. hapla*

The conducted research showed a significant effect of the aqueous extracts of the legume seeds on limiting the motility in the J2 stage of *M. hapla* ($P < 0.0001$) (Tables 2 and 3). The most numerous immotile individuals were observed at a temperature of 21 °C ($P < 0.0001$), in the variant using the soil filtrate from the tomato root ball with tomato root diffusates ($P < 0.0001$).

The invasive larvae of the northern root-knot nematode remained motile in the solution of the seed extract of all the tested species and cultivars of the genus *Lupinus* and the seeds of both *V. faba* cultivars, prepared both in water and in the filtrate of tomato soil, at both temperatures. The J2s remained motile in both control variants as well.

At 10 °C, after 24 h, immotile individuals were observed in the J2+S+H20 variant with the *M. sativa*, *M. albus*, *T. pratense* and *T. repens* seeds.

Plant Protection Science, 60, 2024 (2): 193–206

https://doi.org/10.17221/17/2024-PPS

Table 2. Immobility of the J2 stage of *Meloidogyne hapla* immersed in seed diffusates of selected legume plants at 10, 17 and 21 °C after 24 h

Cultivars	J2 stage immersed in water; control 1				J2stage immersed in seeds diffusates of plants in water				J2 stage immersed in root diffusates of *Solanum lycopersicum*; control 2				J2 stage immersed in seeds diffusates of plants in root diffusates of *Solanum lycopersicum*			
	J2 + H_2O*				J2 + S + H_2O				J2 + RD				J2 + S + RD			
	10 °C	17 °C	21 °C	Mean	10 °C	17 °C	21 °C	Mean	10 °C	17 °C	21 °C	Mean	10 °C	17 °C	21 °C	Mean
GAGOF* cv. Risa	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	15.17^{e}	5.06^{hi}
LOTCO cv. Skrzeszowicka	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	1.67^{klm}	0.56^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	29.20^{a}	28.17^{a}	19.11^{a}
LUPAL	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}
LUPAN cv. Oskar	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}
LUPAN cv. Roland	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}
LUPLU cv. Parys	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}
MEDSA cv. Blue Moon	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.33^{m}	0.00^{m}	10.50^{fg}	3.61^{ij}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	30.00^{a}	17.50^{cde}	15.83^{b}
MEDVA cv. Radius	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	1.8^{klm}	17.50^{cde}	6.44^{h}
MEUAL	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	11.00^{fg}	0.17^{lm}	27.17^{ab}	12.78^{c}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	2.17^{klm}	6.5^{hi}	30.00^{a}	12.89^{c}
MEUOF cv. Pallas	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	19.33^{c}	6.61^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	6.8^{hi}	28.00^{a}	11.61^{cd}
ONBVI cv. Taja	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.50^{lm}	0.00^{m}	0.17^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	29.5^{a}	24.50^{b}	19.83^{a}
OROSA cv. Emena	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.83^{lm}	0.83^{lm}	8.44^{g}
TRFPR cv. Nike	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	4.33^{ijk}	3.00^{jkl}	3.00^{jkl}	12.44^{c}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	30.0^{a}	30.00^{a}	20.00^{a}
TRFRE cv. Grassland Huia	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	2.50^{jlm}	5.30^{ij}	18.33^{cd}	8.72^{g}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	1.63^{klm}	30.00^{a}	10.56^{de}
VICFM cv. Granit	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0^{m}	0.00^{m}	0.00^{k}
VICFM cv. Albus	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0^{m}	0.00^{m}	0.00^{k}
VICSA cv. Ina	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	8.50^{gh}	8.67^{gh}	9.39^{efg}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	12.0^{f}	16.83^{de}	10.39^{def}
VICSA cv. Jaga	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	2.33^{klm}	8.50^{gh}	3.61^{ij}	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{k}	0.00^{m}	0.33^{lm}	7.17^{hi}	2.500^{j}
Mean	0.00^{e}	0.00^{e}	0.00^{e}	–	1.62^{d}	1.10^{d}	6.93^{c}	–	0.00^{g}	0.00^{g}	0.00^{g}	–	3.60^{e}	14.21^{b}	16.45^{a}	–

Means within each column followed by the same letter are not significantly different ($P \leq 0.05$)

Table 3. Immobility of the J2 stage of *Meloidogyne hapla* immersed in seed diffusates of selected legume plants at 10, 17 and 21 °C after 48 h

Cultivars	J2 stage immersed in water; control 1				J2 stage immersed in seeds diffusates of plants in water				J2 stage immersed in root diffusates of *Solanum lycopersicum*; control 2				J2 stage immersed in seeds diffusates of plants in root diffusates of *Solanum lycopersicum*			
	J2 + H_2O*				J2 + S + H_2O				J2 + RD				J2 + S + RD			
	10 °C	17 °C	21 °C	Mean	10 °C	17 °C	21 °C	Mean	10 °C	17 °C	21 °C	Mean	10 °C	17 °C	21 °C	Mean
GAGOF* cv. Risa	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	3.80^{nopq}	10.00^{jkl}	4.61^{ij}
LOTCO cv. Skrzeszowicka	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	1.33^{qr}	17.33^{fg}	30.00^{a}	16.22^{e}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	30.00^{a}	30.00^{a}	30.00^{a}	30.00^{a}
LUPAL	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}
LUPAN cv. Oskar	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}
LUPAN cv. Roland	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}
LUPLU cv. Parys	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	$0^{r}.00$	0.00^{r}
MEDSA cv. Blue Moon	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	1.33^{qr}	6.17^{mno}	25.33^{c}	10.94^{g}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00r	30.00^{a}	24.50^{cd}	18.17^{d}
MEDVA cv. Radius	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	1.50^{pqr}	29.67^{a}	10.39^{g}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	9.33^{kl}	24.00^{cd}	11.11^{g}
MEUAL	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	11.00^{ijk}	30.00^{a}	29.50^{a}	23.50^{b}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	28.17^{ab}	30.00^{a}	30.00^{a}	29.39^{a}
MEUOF cv. Pallas	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	28.33^{ab}	26.33^{bc}	18.22^{d}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.33^{r}	30.00^{a}	30.00^{a}	20.11^{c}
ONBVI cv. Taja	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	12.67^{hij}	3.50^{opq}	5.39^{l}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	30.00^{a}	30.00^{a}	20.00^{c}
OROSA cv. Emena	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	4.17^{nop}	5.67^{nmo}	3.38^{j}
TRFPR cv. Nike	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	10.50^{jkl}	28.50^{ab}	30.00^{a}	23.00^{b}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	30.00^{a}	30.00^{a}	20.00^{c}
TRFRE cv. Grassland Huia	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	2.50^{pqr}	16.80^{g}	19.83^{ef}	13.06^{f}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	30.00^{a}	30.00^{a}	20.00^{c}
VICFM cv. Granit	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}
VICFM cv. Albus	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}
VICSA cv. Ina	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	7.83^{lm}	4.17^{nop}	10.67^{jk}	7.56^{h}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	6.33^{mn}	15.0^{gh}	22.00^{de}	14.44^{f}
VICSA cv. Jaga	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	2.83^{hi}	13.67^{hi}	5.33^{l}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{r}	13.5^{fg}	30.00^{a}	14.50^{f}
Mean	0.00^{g}	0.00^{g}	0.00^{g}	–	1.92^{f}	8.21^{d}	12.14^{c}	–	0.00^{g}	0.00^{g}	0.00^{g}	–	3.60^{e}	14.21^{b}	16.45^{a}	–

Means within each column followed by the same letter are not significantly different ($P \leq 0.05$)

Plant Protection Science, 60, 2024 (2): 193–206

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After 48 h, J2 larvae immersed in water with the *L. corniculatus* and *V. sativa* cv seeds also lost their ability to move. During the first observation date, in the J2+S+RD variant, the immobile J2 stage occurred only when *M. albus* seeds were used, and also after 48 h when the *L. corniculatus*, *M. officinalis* and *V. sativa* cv seeds were used (Tables 2 and 3).

At a temperature of 17 °C, after just 24 h, the J2 stage plants placed in water with the diffused seeds of *M. albus*, *O. viciifolia*, *T. pratense*, *T. repens* and both varieties of *V. sativa* were rendered immotile. After 48 h, the effects of *L. corniculatus*, *M. sativa*, *Medicago varia* and *M. officinalis* were also visible. When the seeds of *L. corniculatus*, *M. sativa*, *Medicago varia*, *M. officinalis* and *O. sativus* were used in combination with the tomato root diffusates, immotile J2 stage individuals were observed already after 24 h. At the second time interval, few immotile J2s also appeared in the variant when the *G. officinalis* seeds were applied.

At a temperature of 21 °C, with the exception of *G. officinalis*, immotile J2 stage individuals were observed when the seeds of all the tested plant species were used, already at the first time interval. The immersion of the J2s in a solution of tomato plant root diffusates resulted in the observed individuals being rendered immotile.

Table 4. Number of galls caused by *Meloidogyne hapla* on the roots of the tomato plants, grown in a substrate with 1%, 5% or 10% of legume seed meal, at temperatures of 17 °C and 21 °C

Seed meal in substrate (%)	Plant species/cultivar	17 °C	21 °C
1	VICSA cv. Ina	0.0^{f}	0.0^{f}
	VICSA cv. Jaga	0.0^{f}	0.0^{f}
	LOTCO cv. Skrzeszowicka	0.0^{f}	0.0^{f}
	ONBVI cv. Taja	0.0^{f}	0.0^{f}
	MEDSA cv. Blue Moon	0.0^{f}	8.8^{d}
	TRFRE cv. Grassland Huia	0.0^{f}	10.4^{cd}
Control	Substrate without seed meal	8.4 ^{cde}	17.2^{ab}
5	VICSA cv. Ina	0.0^{f}	0.0^{f}
	VICSA cv. Jaga	0.0^{f}	0.0^{f}
	LOTCO cv. Skrzeszowicka	0.0^{f}	0.0^{f}
	ONBVI cv. Taja	0.0^{f}	0.0^{f}
	MEDSA cv. Blue Moon	0.0^{f}	2.6^{fg}
	TRFRE cv. Grassland Huia	0.0^{f}	1.2^{f}
Control	Substrate without seed meal	7.2^{de}	17.2^{b}
10	VICSA cv. Ina	0.0^{f}	0.0^{f}
	VICSA cv. Jaga	0.0^{f}	0.0^{f}
	LOTCO cv. Skrzeszowicka	0.0^{f}	0.0^{f}
	ONBVI cv. Taja	0.0^{f}	0.0^{f}
	MEDSA cv. Blue Moon	0.0^{f}	0.0^{f}
	TRFRE cv. Grassland Huia	0.0^{f}	0.0^{f}
Control	Substrate without seed meal	5.6^{e}	19.2^{a}
Temperature	$P < 0.0001$		
Seed meal in substrate (%)	$P = 0.000$		
Plant species	$P < 0.0001$		
Temperature × seed meal in substrate (%)	$P = 0.017$		
Temperature × plant species	$P < 0.0001$		
Seed meal in substrate (%) × plant species	$P = 0.009$		
Temperature × seed meal in substrate (%) × plant species	$P < 0.0001$		

Means within each column followed by the same letter are not significantly different ($P \leq 0.05$)

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Evaluation of the effect of meal from the seeds of selected species of legume plants on the capacity to infect the roots of tomato plants by the J2 stage of *M. hapla* and on the growth of tomato plants

The experiment showed differences in the effect of the temperature, plant species and the content of the seed meal from the tested plants in the substrate on the number of galls (Table 4) and the number of infections in the roots of the test plant (Table 5). This infectivity impacted the development of the tomato plants, which was reflected in their characteristics such as plant weight (Figure 1), height (Figure 2) and number of leaves (Figure 3).

The nematode larvae inhabited the roots of tomato plants in greater numbers at 21 °C than at 17 °C ($P < 0.0001$). Both more galls ($P < 0.0001$) and nematode larvae ($P = 0.005$) were observed in the roots of plants grown with 1% of seed meal added to the substrate than with 5 or 10%. Both the galls ($P < 0.0001$) and larvae ($P < 0.0001$) inside the roots were the most numerous in the control variant. The strongest limiting effect was recorded for both varieties of *V. sativa*, as well as *L. corniculatus* and *O. viciifolia*.

The introduction of seed meal into the soil significantly impacted the fresh weight of the test plant infected with nematodes ($P < 0.0001$) compared to the test plants grown in the substrate

Table 5. Number of infections caused by the northern root-knot nematode *Meloidogyne hapla* in the roots of the tomato plants grown in a substrate with 1%, 5% or 10% of legume seed meal, at temperatures of 17 °C and 21 °C

Seed meal in substrate (%)	Plant/cultivar	17 °C	21 °C
1	VICSA cv. Ina	0.0^{g}	0^{g}
	VICSA cv. Jaga	0.0^{g}	0.0^{g}
	LOTCO cv. Skrzeszowicka	0.0^{g}	0.0^{g}
	ONBVI cv. Taja	0.0^{g}	0.0^{g}
	MEDSA cv. Blue Moon	0.0^{g}	8.8^{cd}
	TRFRE cv. Grassland Huia	0.0^{g}	10.4^{c}
Control	Substrate without seed meal	9.4 ^{cd}	17.2^{b}
5	VICSA cv. Ina	0.0^{g}	0.0^{g}
	VICSA cv. Jaga	0.0^{g}	0.0^{g}
	LOTCO cv. Skrzeszowicka	0.0^{g}	0.0^{g}
	ONBVI cv. Taja	0.0^{g}	0.0^{g}
	MEDSA cv. Blue Moon	2.6^{fg}	2.6^{fg}
	TRFRE cv. Grassland Huia	1.2^{g}	1.2^{g}
Control	Substrate without seed meal	7.2^{de}	17.2^{b}
10	VICSA cv. Ina	0.0^{g}	0.0^{g}
	VICSA cv. Jaga	0.0^{g}	0.0^{g}
	LOTCO cv. Skrzeszowicka	0.0^{g}	0.0^{g}
	ONBVI cv. Taja	0.0^{g}	0.0^{g}
	MEDSA cv. Blue Moon	0.0^{g}	0.0^{g}
	TRFRE cv. Grassland Huia	0.0^{g}	0.0^{g}
Control	Substrate without seed meal	5.6^{ef}	20.8^{a}
Temperature	$P < 0.0001$		
Seed meal in substrate (%)	$P = 0.005$		
Plant species	$P < 0.0001$		
Temperature × seed meal in substrate (%)	$P = 0.007$		
Temperature × plant species	$P < 0.0001$		
Seed meal in substrate (%) × plant species	$P = 0.002$		
Temperature × seed meal in substrate (%) × plant species	$P < 0.0001$		

Means within each column followed by the same letter are not significantly different ($P \leq 0.05$)

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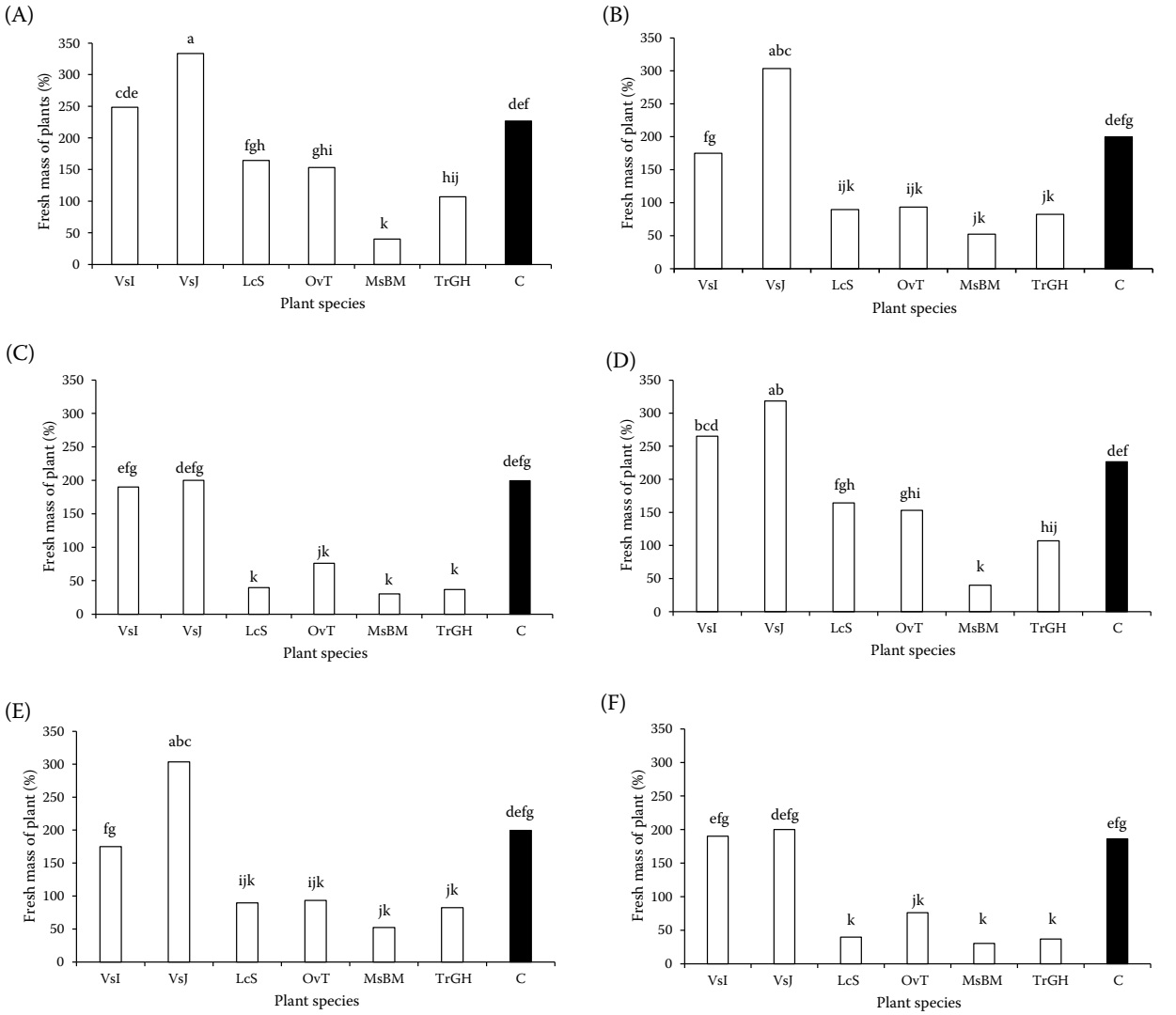

Figure 1. Change in the fresh weight of the tomato plants grown in the substrate with the addition of the seed meal from selected legume species in relation to the initial weight (%)

Means followed by the same letter do not differ ($P = 0.05$). A – temperature 17 °C, seed meal at 1% of the substrate weight; B – temperature 17 °C, seed meal at 5% of the substrate weight; C – temperature 17 °C, seed meal at 10% of the substrate weight; D – temperature 21 °C, seed meal at 1% of the substrate weight; E – temperature 21 °C, seed meal at 5% of the substrate weight; F – temperature 21 °C, seed meal at 10% of the substrate weight. VsL – *Vicia sativa* cv. Ina, VsJ – *Vicia sativa* cv. Jaga, LcS – *Lotus corniculatus* cv. Skrzeszowicka, OvT – *Onobrychis viciifolia* cv. Taja, MsBM – *Medicago sativa* cv. Blue Moon, TrGH – *Trifoilium repens* cv. Grassland Huia; C – Control

without the addition of the seed meal. The results of the root infestation by the northern root-knot nematode are given in Tables 4 and 5. The average weight of the tomato plants decreased with an increase in the proportion of the seed meal in the substrate ($P < 0.0001$), regardless of the ambient temperature ($P = 0.939$). The plant species of the seeds from which the meal was prepared also influenced the change in the weight of the tomato plants ($P < 0.0001$). The addition of the product obtained from the seeds of *L. corniculatus* (5 and 10%), *O. viciifolia*, *M. sativa* cv. Blue Moon, *T. repens* cv. Grassland Huia to the soil negatively affected the fresh weight of the tomato plants. Lower weight values were observed than when the plants were exposed to a nematode infection and remained in the soil with no seed meal added. However, the introduction of *V. sativa* cv. Jaga (1 and 5%) caused an increase in the plant weight compared to the control (Figure 1).

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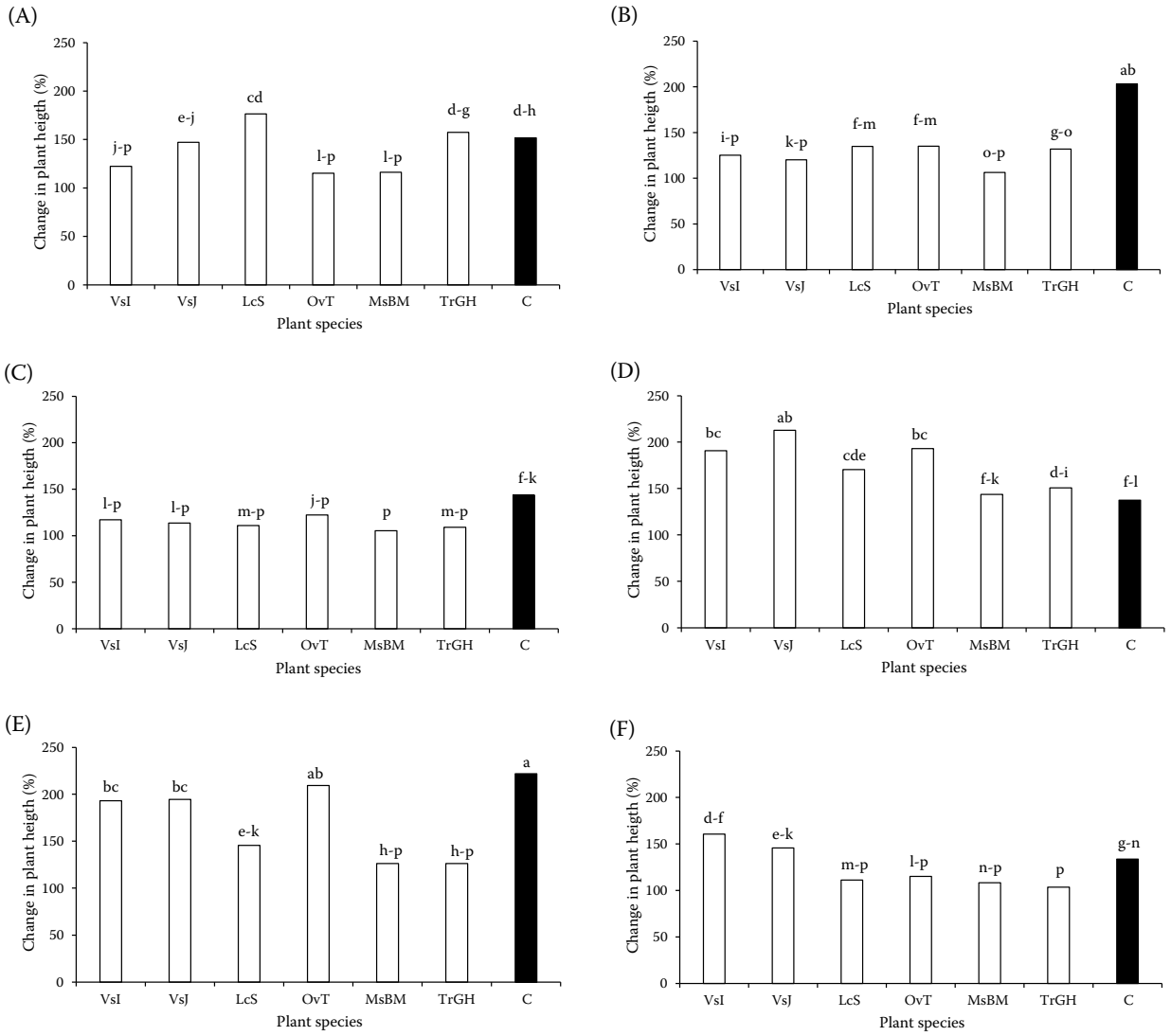

Figure 2. Change in the height of the tomato plants grown in the substrate with the addition of the seed meal from selected legume species in relation to the initial height (%)

Means followed by the same letter do not differ ($P = 0.05$). A – temperature 17 °C, seed meal at 1% of the substrate weight; B – temperature 17 °C, seed meal at 5% of the substrate weight; C – temperature 17 °C, seed meal at 10% of the substrate weight; D – temperature 21 °C, seed meal at 1% of the substrate weight; E – temperature 21 °C, seed meal at 5% of the substrate weight; F – temperature 21 °C, seed meal at 10% of the substrate weight. VsI – *Vicia sativa* cv. Ina, VsJ – *Vicia sativa* cv. Jaga, LcS – *Lotus corniculatus* cv. Skrzeszowicka, OvT – *Onobrychis viciifolia* cv. Taja, MsBM – *Medicago sativa* cv. Blue Moon, TrGH – *Trifoilium repens* cv. Grassland Huia; C – Control

Soil additives had an effect on the growth of tomato plants ($P < 0.0001$). The lowest plant growth was achieved with the addition of the seed meal accounting for 10% of the substrate weight ($P < 0.0001$). At a temperature of 17 °C, except for *V. sativa* cv. Jaga 1%, *L. corniculatus* 1% and *O. vicifolia* 10%, the tomato plants were shorter than the control plants. At a temperature of 21 °C, differences in the height of the tested plants relative to the control were observed, depending on the type and proportion of the seed meal used (Figure 2).

The effect of the temperature, type of seeds and size of the addition on the number of leaves that the plants developed during the experiment was recorded. The plants developed significantly more young leaves at a temperature of 21 °C ($P = 0.015$) with an addition of 1% of the substrate weight ($P = 0.003$). The young leaves were found to be

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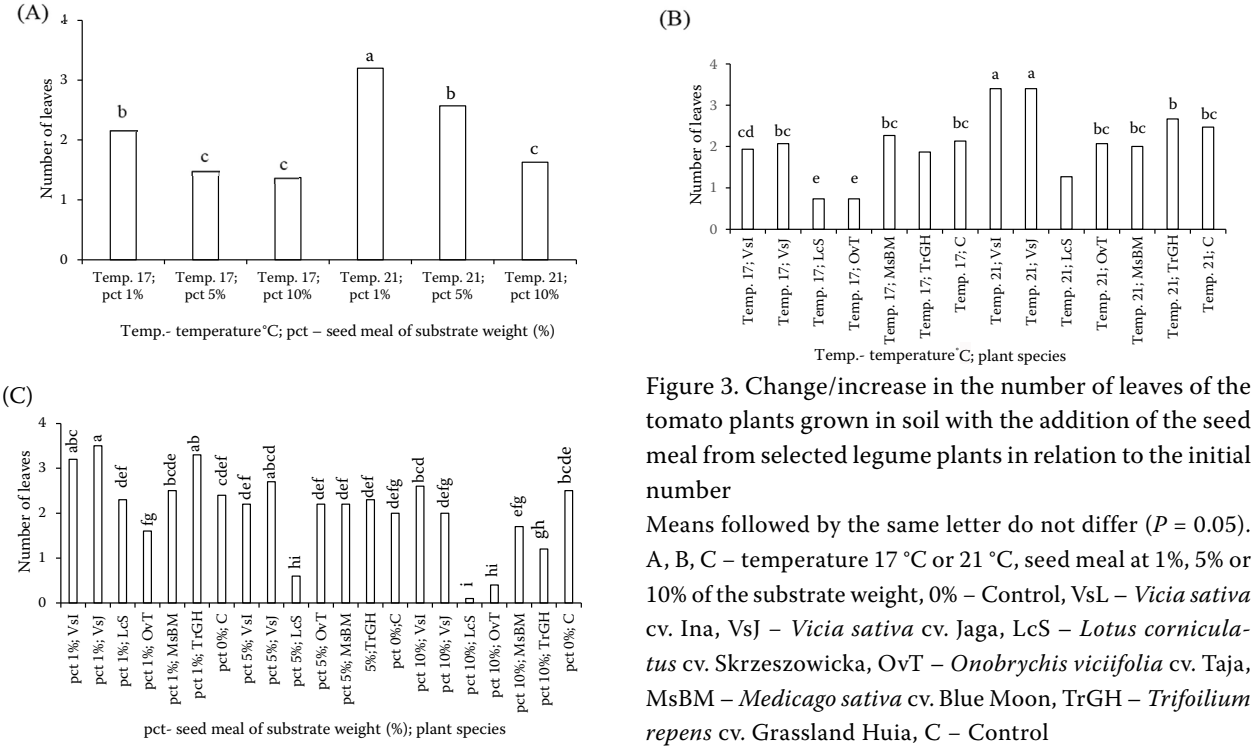

Figure 3. Change/increase in the number of leaves of the tomato plants grown in soil with the addition of the seed meal from selected legume plants in relation to the initial number

Means followed by the same letter do not differ (P = 0.05). A, B, C – temperature 17 °C or 21 °C, seed meal at 1%, 5% or 10% of the substrate weight, 0% – Control, VsL – *Vicia sativa* cv. Ina, VsJ – *Vicia sativa* cv. Jaga, LcS – *Lotus corniculatus* cv. Skrzeszowicka, OvT – *Onobrychis viciifolia* cv. Taja, MsBM – *Medicago sativa* cv. Blue Moon, TrGH – *Trifoilium repens* cv. Grassland Huia, C – Control

most abundant with the addition of *V. sativa* cv. Ina, *V. sativa* cv. Jaga and *T. repens* cv. Grassland Huia (P < 0.0001) (Figure 3).

DISCUSSION

Due to the necessity to limit the chemical control of nematodes/root-knot nematodes, natural ways/methods of combating these nematodes are recommended, based, among other solutions, on the natural properties of plants. These are additives introduced into the soil obtained from fresh or dry plant tissues and seeds (Quilty & Cattle 2011; Renčo 2013). Their purpose is to limit the motility of nematodes in the invasive stage, preventing the infection of root tissues and their transition into further developmental stages. The efficacy of products obtained from legume seeds was demonstrated by Osei et al. (2013) and Bawa et al. (2014). The introduction of seed meal from cowitch seeds (*Mucuna pruriens* L.) into the soil significantly reduced the number of *Meloidogyne* spp. in the soil with yam [*Dioscorea rotundata* (Poir.)] cultivation (Osei et al. 2013). However, in the studies by Bawa et al. (2014), it was demonstrated that application of alcohol extracts from *Parkia biglobosa* (Jacq.) seeds limited the development of *M. incognita* in tomato cultivation. The study assessed the possibilities of using legume seeds to reduce the infectivity of the northern root-knot nematode. Seeds of legume species and varieties used in agriculture in the temperate climate zone were used.

Temperature is an important factor influencing the motor activity of the J2 stage. The J2 stage of the nematode retains the capacity to move between 10 °C and 35 °C, with an optimum between 15 °C and 25 °C (Bird & Wallce 1966; Den Belder & Jansen 1994; Boroş et al. 2018). The observation of immotile J2 individuals at temperatures of 17 °C and 21 °C, which fall within the temperature range of the optimal activity of the northern root-knot nematode, allowed us to assume that the diffusates would also be effective in the soil environment. The temperatures at which the experiment was carried out are those found in the soils of the temperate climate zone which constitutes the geographical range of the nematode and where this species is economically destructive.

The first immotile individuals were observed after just 24 h of contact with the diffusates, which is extremely important from the point of view of protecting crops against the northern root-knot nematode, as the effect of the product introduced into the soil should be quick. The increase in the effectiveness of legume seed diffusates in the pres-

https://doi.org/10.17221/17/2024-PPS

ence of the soil filtrate with root secretions of the test plant might imply a beneficial effect, reinforcing the natural properties of the root zone environment of the nematode host plant. It can be assumed that the diversity of these interactions is the result of the individual interaction of the seed diffusates, soil filtrate and tomato root secretions, resulting from the chemical composition and the microbiological environment of the biological system under study. Similar conclusions were obtained by Dobosz et al. (2023).

Mixing seed meal from selected species and varieties with the substrate resulted in a significant reduction in the number of galls and the number of northern root-knot nematode infections in the tomato roots compared to the control. However, the reduction in the number of infections was, in most cases, accompanied by a deterioration in the plant growth. The phytotoxic effect of additives from alfalfa and clover seeds, as well as trefoil and sainfoin significantly reduced the fresh weight of plants compared to the control. The deterioration of the growth parameters of test plants (tomato) was also observed by Morris and Walker (2002) when dried tissues of *Canavalia ensiformis* (L.) or *Indigofera spicata* Forsskal were applied to the soil.

Previous studies have shown that the soil additives *Delbergia sissoo* and Roxb. ex DC., *Pithocellobium dulce* (Roxb.), *Prosopis juliflora* (Sw) and *Samanea saman* (Jacq.) and *Tamarindus indica* L. (Latif et al. 2014), gum arabic [*Acacia nilotica* (L.)] and locust bean [*Parkia biglobosa* (Jacq.)] (Eche & Okafor 2020) as well as *M. sativa* (D'Addabbo et al. 2020) can, by reducing the infectivity of northern root-knot nematodes, improve the growth and development of plants, through the effect of a fertilising value, while enriching them with organic matter, improving the soil fertility. The results reported in literature regarding *M. sativa* differ significantly from our own described observations. A previous study also showed an unfavourable effect of *M. sativa* when grown as a forecrop for tomatoes (Dobosz & Krawczyk 2021). The discrepancy in results may be explained by differences between the plant varieties used, test conditions and the type of material used (dry biomass vs. seed meal). The presented studies, however, showed a favourable effect of vetch seed meal applied in low (1% and 5%) doses, as this species is not a suitable host plant for the northern root-knot nematode (Dobosz & Krawczyk 2019). In addition to the positive impact on the growth and development, it has also been noticed that products made from plant tissues have a beneficial effect on the microbial richness of the soil (Jaffee 2006; Chauvin et al. 2015; Chen et al. 2019) and the diversity of bacterivorous and fungivorous nematode communities in the soil (Jaffee 2006; Renčo 2021). However, in this study, the impact on the biological health of the substrate was not examined.

CONCLUSION

The described studies, performed under controlled conditions, highlighted the effect of legume seeds commonly grown in the temperate climate zone on the motility and infectivity of the J2 stage of *M. hapla*. Diffusates from the tested seeds limited the motility of the J2 stage, and the speed and intensity of the phenomenon depended on the temperature and environment of the root diffusates of the test plant - the tomato.

The addition of *V. sativa* cv. Ina, *V. sativa* cv. Jaga, *L. corniculatus* cv. Skrzeszowicka, *O. sativus* cv. Emena, *M. sativa* cv. Blue Moon and *T. repens* Grassland Huia seed meal to the tomato growing substrate resulted in a reduced number of galls and J2 stage infections in the tomato roots. The admixture of *V. sativa* cv. Ina, *V. sativa* cv. Jaga also showed a positive effect on the growth of plants infested with the nematode.

The obtained results imply that it is advisable to expand the scope of research to include other economically important crops damaged by the northern root-knot nematode.

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Received: January 26, 2024
Accepted: March 11, 2024
Published online: May 9, 2024